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(54) Title: HIGH CONCENTRATION IMMUNOGLOBULIN PREPARATION AND METHOD FOR ITS PRODUCTION

(57) Abstract

The present invention provides intravenously tolerable immunoglobulin preparations which are stable over prolonged storage periods. The present invention also provides a method for the productionof a clinically acceptable immunoglobulin preparation. The method comprises the following steps: (1) obtaining an immunoglobulin solution; (2) subjecting the immunoglobulin solution to conditions which enhance the formation of immunoglobulin aggregates to form an immunoglobulin aggregate containing solution; and (3) removing the immunoglobulin aggregates from the immunoglobulin aggregate containing solution to obtain an immunoglobulin preparation substantially free of immunoglobulin aggregates.

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High Concentration Immunoglobulin Preparation and Method for its Production

FIELD OF INVENTION

The present invention relates to a stable, high concentration immunoglobulin preparation and to a method of producing such an immunoglobulin preparation.

BACKGROUND OF THE INVENTION

Human plasma has long been a source of therapeutic proteins for use as replacement therapy for individuals suffering from various haematological conditions. For example, a deficiency in immunoglobulins results in a condition known as agammaglobulinaemia which results in a predisposition by the sufferer to infections. This condition may be treated by the administration of IgG purified from the pooled plasma of healthy individuals. A shortcoming of this approach to therapy is that the immunoglobulin preparation must be relatively free of polymeric immunoglobulin species such as aggregates which following administration may induce severe adverse reactions.

It has been observed that immunoglobulin aggregates result from the use of destabilizing conditions during the manufacture of the plasma derived IgG product. These protein destabilizing conditions include the use of ethanol to isolate the product and heat to virally inactivate the isolated IgG. Once unfolded a subpopulation of the IgG molecules may refold to form various polymeric species including trimers, tetramers and aggregates. The formation of aggregated species appears to be cumulative with time. Thus it is imperative that IgG species which have aggregated or are prone to aggregate are removed prior to dispensing. This removal of aggregates typically occurs just prior to formulation so that aggregates formed throughout the process are removed.

The present inventors have now surprisingly found that if an immunoglobulin solution is exposed to conditions to deliberately enhance the formation of aggregates, and the aggregates are then removed, an immunoglobulin preparation is obtained which is stable at high concentrations for prolonged periods. The present inventors have also made the observation that this clarification of the immunoglobulin solution to

remove aggregates increases the stability and thus the clinical acceptability of the product. In fact the immunoglobulin solution prepared by this method is sufficiently stable that it may be formulated into a product which is room temperature stable and capable of being administered intramuscularly, intravenously or subcutaneously.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention consists in a clinically acceptable immunoglobulin preparation, the preparation comprising about 5% to about 25% (w/v) immunoglobulin and an osmolality agent and /or a stabilizer and having a pH of about 5.0 to about 8.0, wherein the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 3 months is less than 2% (w/v).

In a second aspect the present invention consists in a clinically acceptable immunoglobulin preparation, the preparation comprising about 5% to about 25% (w/v) immunoglobulin and an osmolality agent and /or a stabilizer and having a pH of about 4.0 to about 8.0, wherein the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 4°C for 3 months is less than 1% (w/v).

In a preferred embodiment the concentration of immunoglobulin is about 10% to about 25% (w/v), preferably about 20% (w/v) or about 25% (w/v).

It is preferred that the pH of the immunoglobulin preparation is about 5.0 to about 6.0, more preferably about 5.5. This is particularly so when the immunoglobulin preparation is to be stored for prolonged periods at room temperature, eg 27°C.

The preparation of the present invention will include an osmolality agent such that the osmolality of preparation is suitable for administration. This osmolality agent may also be a compound which stabilizes the immunoglobulin. Stabilizers are well known in the art and include saccharides, such as sucrose, maltose and glucose, sugar alcohols, such as sorbitol and mannitol, and amino acids.

In a preferred embodiment of the present invention the preparation further comprises glycine.

In other preferred embodiments the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 6 months is less than 2% (w/v), and more preferably the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 9 months is less than 2% (w/v).

It is also preferred that the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 6 months is less than 1% (w/v).

In other preferred embodiments the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 4°C for 6 months is less than 1% (w/v), and preferably the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 4°C for 9 months is less than 1% (w/v).

In a third aspect the present invention consists in a method for the production of a clinically acceptable immunoglobulin preparation, the method comprising the following steps;-

(1) obtaining an immunoglobulin solution;

(2) subjecting the immunoglobulin solution to conditions which enhance the formation of immunoglobulin aggregates to form an immunoglobulin aggregate containing solution; and

(3) removing the immunoglobulin aggregates from the immunoglobulin aggregate containing solution to obtain an immunoglobulin preparation substantially free of immunoglobulin aggregates.

By "enhance the formation of immunoglobulin aggregates" we mean the inclusion or modification of a step in the production of an immunoglobulin preparation to deliberately cause or promote the formation of immunoglobulin aggregates.

In a preferred embodiment step (2) comprises incubating the immunoglobulin solution at a pH of about 5.8 to about 8.0 at temperature of about 4° to about 27°C for at least about 6 hours. It is preferred that the immunoglobulin solution is incubated at a pH of about 6.8 for about 12 hours.

In a further preferred embodiment the aggregates are removed from the aggregate containing solution by filtration, precipitation or size exclusion chromatography.

In one preferred embodiment the aggregates are removed from the aggregate containing solution by precipitation using a precipitation enhancing agent. A preferred precipitation enhancing agent is polyethylene glycol. Preferably the precipitation is performed at a protein concentration of about 2 to about 10% (w/v) and a polyethylene glycol concentration of about 6 to about 12% w/w and at a pH of about 6 to about 8.

As will be understood by those skilled in this field numerous conditions can be used to promote aggregation. These include manipulation of pH together with incubation for various periods of time at various temperatures. In addition incubation at elevated temperatures, eg 37°C to 42°C, and/or increasing ionic strength can be used to cause aggregate formation.

The aggregates formed may be removed using any of a number of well known techniques, however it is presently preferred that the aggregates are removed by filtration or precipitation, optionally using a precipitation enhancing agent such as PEG.

For example the aggregates may be removed by membrane filtration using a filter of exclusion limits greater than 300,000 kDa. Alternatively the aggregates may be removed using size exclusion chromatography eg using a resin of exclusion limits of 10,000 - 1,5000,000.

It may be advantageous to remove the aggregates by membrane filtration in which the membrane filtration serves to remove viruses.

It is believed that the present invention will also have applicability for a range of protein preparations other than immunoglobulins, where the removal of protein aggregates is desirable.

As will be understood by those working in this field the preparation of the present invention may be administered intravenously, intramuscularly or subcutaneously.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

DETAILED DESCRIPTION

In order that the nature of the present invention may be more readily understood preferred forms thereof will now be described with reference to the following non-limiting examples.

Example 1 Stability of 10% IgG preparations following aggregate removal by membrane filtration

Starting material for this experiment was an IgG preparation produced by a combination of Cohn fractionation and chromatographic methods. The preparation had been pasteurised in the presence of sucrose, followed by diafiltration against water, the final protein concentration being approximately 2% (w/v). The solution was then adjusted to pH 6.8 and maintained at this pH for 12hrs. Prior to membrane passage the solution was adjusted to pH 4.2. The membrane was a Millipore PLCXK virus filter membrane (surface area = 0.1 m^2). Five hundred and fifty millilitres of the IgG preparation was processed through the filter under the following conditions: transmembrane pressure = 4.5 psi, cross flow rate = 500 mL/min. Water was added to the retentate pool at a rate of 40 mL/min to maintain a constant protein concentration level in the permeate. At the conclusion of the run water was added to the retentate in 500 mL volumes to increase IgG recovery across the membrane.

Samples of the starting material, final permeate pool, and final retentate pool were analysed by high performance liquid chromatography (HPLC) for determination of aggregate content (Table 1.)

Table 1. Determination of monomer, dimer, and aggregate content of IgG before and after ultrafiltration across a PLCXK 1,000 kDa membrane.

Sample	aggregate (%)	Dimer (%)	monomer (%)
Starting material	0.6	4.9	93.3
Final retentate	0.8	4.4	94.4
Permeate pool	0.0	2.0	97.4

The process has thus resulted in complete removal of aggregate IgG.

The filtered material was then diafiltered against PFW and concentrated to 10% w/v using a Amicon 30kDa membrane. Following concentration the solution was formulated to 10% w/v IgG containing 0.2 M glycine pH 5.5. Formulated samples were then incubated at various temperatures and stability was assessed over time by comparing protein composition with that of non-filtered solutions (Table 2).

Table 2 Stability of 10% IgG solutions formulated to pH 5.5 following filtration to remove aggregates

Protein Composition	Time 0 Month 4°C		Time 1 Month 4°C		Time 9 Months 4°C		Time 1 Month 27°C		Time 9 Months 27°C	
	F*	C**	F	C	F	C	F	C	F	C
Aggregate	0.0	0.3	0.0	0.3	0.1	1.1	0.2	1.76	1.2	4.8
Dimer	3.8	7.2	4.8	7.8	6.4	9.4	4.5	7.7	5.5	6.2
Monomer	93.6	90.9	93.8	90.9	90.9	88.1	93.4	89.3	90.7	87.4
Fragments	2.6	1.6	1.7	0.9	2.5	1.4	1.0	1.2	2.6	1.7

F* Filtered material

C** Non-Filtered material

From Table 2 it can be seen that the filtered solution when compared to the non-treated solution remains stable even at higher temperatures.

Example 2 Stability of 16% IgG preparations following aggregate removal by membrane filtration

A similar procedure as described in Example 1 was used to prepare an aggregate depleted IgG solution. The solution was then concentrated and formulated to 16% w/v containing 0.25 Glycine pH 4.25 or 5.5. Samples of the final product were placed on stability trial and aggregate content determined (Tables 3a –3d.).

Table 3a: 16% IgG, pH 4.25, 4 °C

		Storage Time (Months)						
		0	1	3	4	5	6	8
Protein Composition	Aggregate	0.1	0.1	0.1	0.1	0.2	0.1	0.1
	Dimer	3.3	2.7	2.6	2.0	4.0	2.6	2.9
	Monomer	95.0	95.6	96.0	94.8	94.4	96.0	95.5
	Fragments	1.8	1.6	1.5	3.2	1.5	1.4	1.6

Table 3b: 16% IgG, pH 4.25, 27 °C

		Storage Time (Months)						
		0	1	3	4	5	6	8
Protein Composition	Aggregate	0.1	1.6	2.2	3.1	4.9	5.1	9.1
	Dimer	3.3	3.1	3.2	3.3	4.3	4.1	4.8
	Monomer	95.0	93.4	91.3	88.9	86.9	86.9	81.3
	Fragments	1.8	1.9	3.4	4.7	3.8	3.9	4.8

Table 3c: 16% IgG, pH 5.5, 4 °C

		Storage Time (Months)						
		0	1	3	4	5	6	8
Protein Composition	Aggregate	0.1	0.2	0.3	0.2	0.5	0.3	0.5
	Dimer	6.2	7.1	7.5	6.4	9.3	6.1	7.8
	Monomer	90.6	91.1	90.7	90.0	88.8	92.2	90.3
	Fragments	3.1	1.5	1.4	3.3	1.3	1.4	1.4

Table 3d: 16% IgG, pH 5.5, 27 °C

		Storage Time (Months)						
		0	1	3	4	5	6	8
Protein Composition	Aggregate	0.1	0.3	0.4	0.5	0.5	0.6	0.6
	Dimer	6.2	5.4	5.3	4.6	5.6	4.8	4.9
	Monomer	90.5	92.5	91.9	91.5	91.8	92.	91.8
	Fragments	3.1	1.7	2.3	3.3	2.0	2.2	2.6

Example 3 Stability of 16 % IgG preparations following aggregate removal by PEG precipitation

Starting material for this experiment was an IgG preparation produced by a combination of Cohn fractionation and chromatographic methods. The preparation had been pasteurised in the presence of sucrose and formulated for low pH incubation by concentrating to 5% w/v, adjusting the pH to 4.2 and incubating at 27°C for 14 days. Following incubation the material was adjusted to pH 6.8 and maintained at this pH for 12hrs. PEG 1000 was then added until a final concentration of 10% w/w PEG was achieved. The material was then left to settle for 1 hour and centrifuged. The supernatant was removed and analysed for aggregate content (Table 4).

Table 4. Determination of monomer, dimer, and aggregate content of IgG before and after PEG treatment.

Sample	Aggregate (%)	dimer (%)	Monomer (%)
Starting material pH 6.8	1.1	6.6	91.9
PEG precipitated material	0.0	6.3	92.0

The supernatant was then diafiltered against 8 exchanges of PFW to remove residual PEG. Following diafiltration the IgG solution was concentrated and formulated to 16% w/v containing 0.25M glycine, pH 4.25, 5.5 or 6.8. The material was then dispensed and placed on stability trials where it was analysed for aggregate content (Table 5a and 5f).

Table 5a. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 4.25 following storage at 4°C.

Protein Composition	Time 0	1 Month PEG Treated	4 Months PEG Treated	8 Months PEG Treated	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.01	0.12	0.0	0.1	0.14	0.42	0.15	0.61
Dimer	1.63	3.01	2.09	3.44	2.52	4.28	2.16	4.43
Monomer	98.37	96.87	97.91	95.22	97.33	95.30	97.69	93.85

Table 5b. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 4.25 following storage at 27°C.

Protein Composition	Time 0	1 Month PEG	4 Months PEG	8 Months PEG	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.01	6.44	9.65	14.89	0.14	6.71	10.22	19.37
Dimer	1.63	3.84	2.10	4.01	2.52	4.50	2.03	3.65
Monomer	98.37	89.71	86.99	64.03	97.33	88.79	86.25	60.22

Table 5c. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 5.5 following storage at 4°C.

Protein Composition	Time 0	1 Month PEG Treated	4 Months PEG Treated	8 Months PEG Treated	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.06	0.21	0.12	0.15	0.26	1.46	0.94	1.93
Dimer	2.01	5.47	5.83	6.58	3.56	7.32	6.71	7.21
Monomer	97.93	94.32	94.04	92.26	96.18	91.21	92.35	89.95

Table 5d. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 5.5 following storage at 27°C.

Protein Composition	Time 0	1 Month PEG	4 Months PEG	8 Months PEG	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.06	0.34	0.35	0.99	0.26	6.71	4.36	5.94
Dimer	2.01	5.82	5.57	8.11	3.56	4.50	5.87	7.05
Monomer	97.93	93.84	94.08	88.94	96.18	89.71	89.77	85.01

Table 5e. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 6.8 following storage at 4°C.

Protein Composition	Time 0	1 Month PEG Treated	4 Months PEG Treated	8 Months PEG Treated	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.0	0.26	0.20	0.21	1.58	4.12	4.06	3.84
Dimer	5.22	8.42	9.25	8.89	6.58	9.69	10.56	10.18
Monomer	94.78	91.32	90.55	89.86	91.84	86.18	85.37	84.97

Table 5f. Determination of monomer, dimer, and aggregate content of PEG treated IgG 16% w/v, pH 6.8 following storage at 27°C.

Protein Composition	Time 0	1 Month PEG	4 Months PEG	8 Months PEG	Time 0 Control	1 Month Control	4 Months Control	8 Months Control
Aggregate	0.0	0.66	0.50	0.39	1.58	6.33	0.82	0.15
Dimer	5.22	7.84	6.23	6.73	6.58	7.35	6.58	7.22
Monomer	94.78	91.12	90.79	71.77	91.84	86.22	91.24	75.07

Example 4 Stability of 10% IgG preparations following aggregate removal by PEG precipitation

A similar procedure as described in example 3 was used to prepare the PEG treated IgG solution. Following aggregate removal the IgG solution was concentrated and formulated to 10% protein w/v containing 0.2M glycine, pH 4.25 or 5.5. The material was then dispensed and placed on stability trials where it was analysed for aggregate content. The results obtained are set out in Table 6a & b.

Table 6a Determination of monomer, dimer, and aggregate content of PEG treated IgG 10% w/v, pH 4.25 following storage at 4° and 27°C.

Storage Conditions	Aggregate %	Dimer %	Monomer %
1 Month PEG Treated 4°C	0.18	3.33	96.49
4 Months PEG Treated 4°C	0.38	4.33	95.30
8 Months PEG Treated 4°C	0.17	5.29	94.54
12 Months PEG Treated 4°C	0.17	5.29	93.70
1 Month PEG Treated 27°C	1.01	4.01	94.98
4 Months PEG Treated 27°C	9.55	4.11	83.16
8 Months PEG Treated 27°C	13.74	4.66	63.92
12 Months PEG Treated 27°C	17.62	5.18	54.55

Table 6b Determination of monomer, dimer, and aggregate content of PEG treated IgG 10% w/v, pH 5.5 following storage at 4° and 27°C.

Storage Conditions	Aggregate %	Dimer %	Monomer %
1 Month PEG Treated 4°C	0.21	8.73	91.07
4 Months PEG Treated 4°C	0.35	9.36	90.26
8 Months PEG Treated 4°C	0.29	12.21	87.49
12 Months PEG Treated 4°C	0.53	11.81	86.99
1 Month PEG Treated 27°C	0.41	9.54	90.06
4 Months PEG Treated 27°C	0.63	10.14	89.23
8 Months PEG Treated 27°C	0.88	10.43	87.34
12 Months PEG Treated 27°C	1.09	11.20	85.79

Example 5 Stability of 20 and 25% IgG preparations following aggregate removal by PEG precipitation

A similar procedure as described in example 3 was used to prepare the PEG treated IgG solution. Following aggregate removal the IgG solution was concentrated and formulated to 20 and 25% protein w/v containing 0.3M glycine, pH 5.5. The material was then dispensed and placed on stability trials where it was analysed for aggregate content (Table 7a & 7b).

From Tables 5a - 7b it can be seen that the PEG treated material when compared to the non-treated solutions have significantly lower aggregate contents even after storage at higher temperatures.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Table 7a. Determination of monomer, dimer, and aggregate content of PEG treated IgG 20% w/v, pH 5.5 following storage at 4°, 27°, 32° & 37°C for 1 month.

Protein Composition	Time 0 PEG Treated	PEG Treated 4°C	PEG Treated 27°C	PEG Treated 32°C	PEG Treated 37°C	Time 0 Control	Control 4°C	Control 27°C	Control 32°C	Control 37°C
Aggregate	0.17	0.24	0.33	0.40	0.78	1.18	1.65	2.87	2.99	3.88
Dimer	7.13	8.74	8.33	8.13	7.57	6.59	7.60	6.17	6.44	5.89
Monomer	92.7	90.24	90.4	90.55	90.5	92.23	90.10	90.24	89.74	89.2

Table 7b. Determination of monomer, dimer, and aggregate content of PEG treated IgG 25% w/v, pH 5.5 following storage at 4°, 27°, 32° & 37°C for 1 month.

Protein Composition	Time 0 PEG Treated	PEG Treated 4°C	PEG Treated 27°C	PEG Treated 32°C	PEG Treated 37°C	Time 0 Control	Control 4°C	Control 27°C	Control 32°C	Control 37°C
Aggregate	0.19	0.30	0.43	0.07	1.20	1.36	1.99	3.12	3.32	4.03
Dimer	7.98	9.67	9.44	8.40	8.97	6.43	7.33	6.65	6.30	6.0
Monomer	91.84	89.16	89.19	88.84	88.53	92.21	90.14	89.43	89.50	88.95

CLAIMS:-

1. A clinically acceptable immunoglobulin preparation, the preparation comprising about 5% to about 25% (w/v) immunoglobulin and an osmolality agent and /or a stabilizer and having a pH of about 5.0 to about 8.0, wherein the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 3 months is less than 2% (w/v).

2. An immunoglobulin preparation as claimed in claim 1 in which the concentration of immunoglobulin is about 10% to about 25% (w/v).

3. An immunoglobulin preparation as claimed in claim 1 in which the concentration of immunoglobulin is about 20% (w/v).

4. An immunoglobulin preparation as claimed in claim 1 in which the concentration of immunoglobulin is about 25% (w/v).

5. An immunoglobulin preparation as claimed in any one of claims 1 to 4 in which the preparation has a pH of about 5.0 to about 6.0.

6. An immunoglobulin preparation as claimed in claim 5 in which the preparation has a pH of about 5.5.

7. An immunoglobulin preparation as claimed in claim 5 or claim 6 in which the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 6 months is less than 2% (w/v).

8. An immunoglobulin preparation as claimed in any one of claims 5 to 7 in which the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 9 months is less than 2% (w/v).

9. An immunoglobulin preparation as claimed in any one of claims 5 to 6 in which the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 27°C for 6 months is less than 1% (w/v).

10. An immunoglobulin preparation as claimed in any one of claims 1 to 9 in which the preparation further comprises glycine.

11. A clinically acceptable immunoglobulin preparation, the preparation comprising about 5% to about 25% (w/v) immunoglobulin and an osmolality agent and /or a stabilizer and having a pH of about 4.0 to about 8.0, wherein the concentration of immunoglobulin aggregates present in the

immunoglobulin preparation after storage at 4°C for 3 months is less than 1% (w/v).

12. An immunoglobulin preparation as claimed in claim 11 in which the concentration of immunoglobulin is about 10% to about 25% (w/v).

13. An immunoglobulin preparation as claimed in claim 11 in which the concentration of immunoglobulin is about 20% (w/v).

14. An immunoglobulin preparation as claimed in claim 11 in which the concentration of immunoglobulin is about 25% (w/v).

15. An immunoglobulin preparation as claimed in any one of claims 11 to 14 in which the preparation has a pH of about 5.0 to about 6.0.

16. An immunoglobulin preparation as claimed in claim 15 in which the preparation has a pH of about 5.5.

17. An immunoglobulin preparation as claimed in any one of claims 11 to 16 in which the preparation further comprises glycine.

18. An immunoglobulin preparation as claimed in any one of claims 11 to 17 in which the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 4°C for 6 months is less than 1% (w/v).

19. An immunoglobulin preparation as claimed in any one of claims 11 to 18 in which the concentration of immunoglobulin aggregates present in the immunoglobulin preparation after storage at 4°C for 9 months is less than 1% (w/v).

20. A method for the production of a clinically acceptable immunoglobulin preparation, the method comprising the following steps;-

(1) obtaining an immunoglobulin solution;

(2) subjecting the immunoglobulin solution to conditions which enhance the formation of immunoglobulin aggregates to form an immunoglobulin aggregate containing solution; and

(3) removing the immunoglobulin aggregates from the immunoglobulin aggregate containing solution to obtain an immunoglobulin preparation substantially free of immunoglobulin aggregates.

21. A method as claimed in claim 20 in which step (2) comprises incubating the immunoglobulin solution at a pH of about 5.8 to about 8.0 at temperature of about 4° to about 27°C for at least about 6 hours.

22. A method as claimed in claim 20 in which the immunoglobulin solution is incubated at a pH of about 6.8 for about 12 hours.

23. A method as claimed in any one of claims 20 to 22 in which the aggregates are removed from the aggregate containing solution by filtration, precipitation or size exclusion chromatography.

24. A method as claimed in claim 23 in which the aggregates are removed from the aggregate containing solution by precipitation using a precipitation enhancing agent.

25. A method as claimed in claim 24 in which the precipitation enhancing agent is polyethylene glycol.

26. A method as claimed in claim 25 in which the precipitation is performed at a protein concentration of about 2 to about 10% (w/v) and a polyethylene glycol concentration of about 6 to about 12% w/w and at a pH of about 6 to about 8.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00433

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 039/395; A61P 007/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K, SEARCH TERMS AS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CA ONLINE (STN): immunoglobulin AND (stab: OR concentrat: OR storage OR pH) AND (haemato: OR blood OR plasma) AND immunoglobulin()aggregat:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU-A-34297/97 (CSL LIMITED) 29 January 1998 See whole document	1, 5-9, 11, 15-16, 18-19
X	US 4,845,199A (HIRAO ET AL.) 4 July 1989 See whole document	1-9, 11-16, 18-19
X	US 4,597,966A (ZOLTON ET AL.) 1 July 1986 See whole document	1, 5, 7-9, 11, 15, 18-19

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 2000	07 JUL 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer MICHAEL GRIEVE Telephone No : (02) 6283 2267

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00433

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,880,913A (DOLESCHEL ET AL.) 14 November 1989 See whole document	1, 20, 19-24
X	EP 0123029A (SCHWAB & CO. GES.M.B.H.) 31 October 1984 See whole document	20, 19, 21-22
P,X	WO 99/64462A (STATENS SERUM INSTITUT) 16 December 1999 See whole document	20, 19, 21-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00433

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	34297/97	WO	9803550	EP	954534	CA	2261291
US	4845199	CA	1310267	EP	253313	JP	63146832
US	4597966	AU	51892/86	CA	1285225	EP	187712
		JP	61218528				
US	4880913	DE	3641115	EP	270025		
EP	123029	AT	2126/83	DE	3310150	JP	59176215
WO	9964462	AU	42572/99				

END OF ANNEX